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DIFFERENTIAL ROLES OF M2 AND M3 MUSCARINIC RECEPTOR SUBTYPES IN MODULATION OF BLADDER AFFERENT ACTIVITY

Hypothesis / aims of study

It has recently been reported that the urothelium expresses various receptors including muscarinic receptors, releases various neurotransmitters in response to sensory stimuli, and thereby modulates activity of bladder afferent pathways (1). It is well known that detrusor contraction is directly mediated by M3 muscarinic receptor subtypes on the detrusor while M2 muscarinic receptor subtypes indirectly modulate the contraction (2). However, the differential roles of M2 and M3 muscarinic receptor subtypes in local cholinergic modulation of urothelial-afferent interactions remain unclear. We therefore investigated the effects of various muscarinic receptor antagonists including selective M2 or M3 muscarinic receptor blockers on bladder overactivity induced by local muscarinic receptor stimulation.

Study design, materials and methods

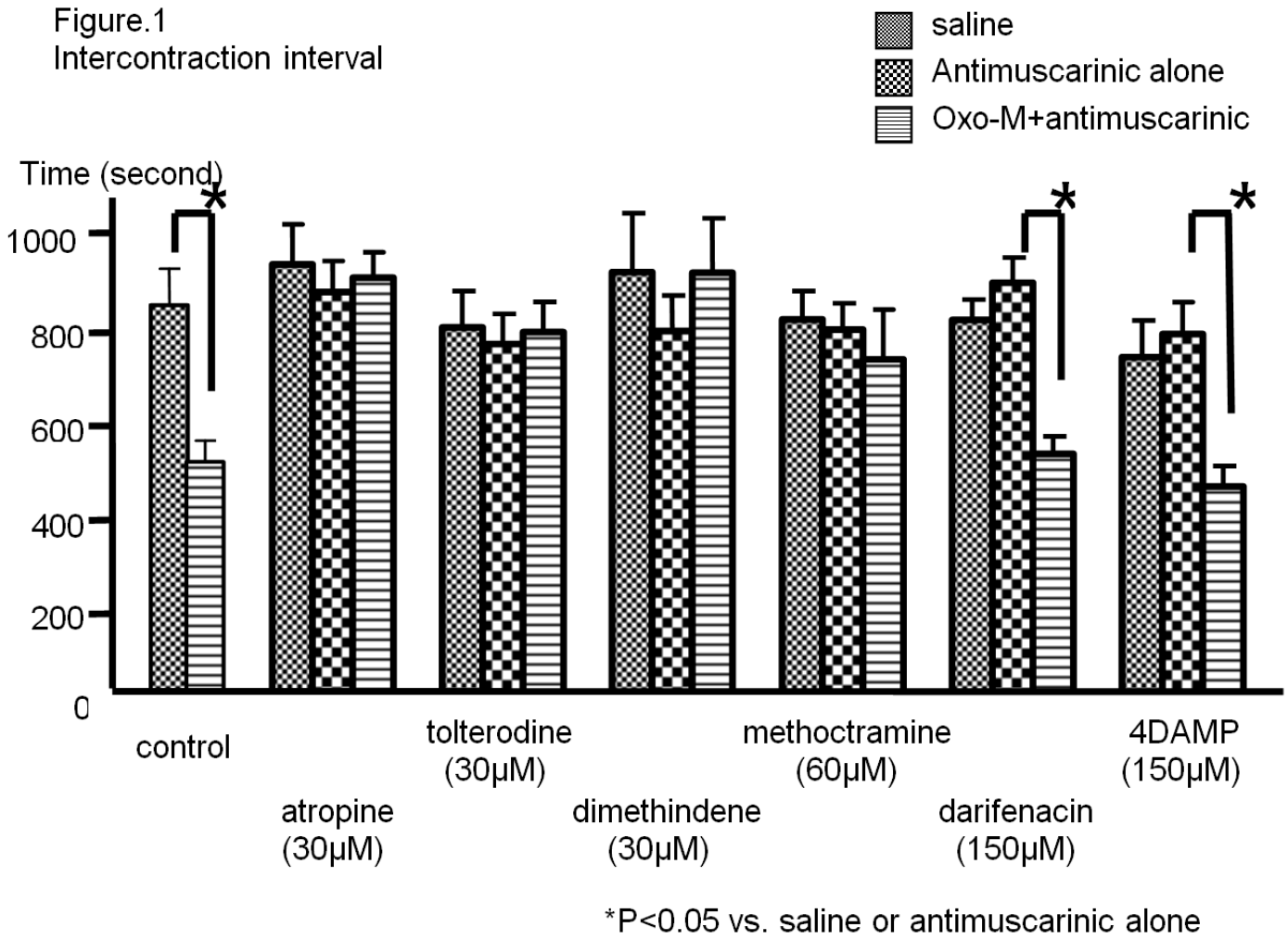
Animal: Female Sprague-Dawley rats weighing 250 to 300 g were used.

Cystometry: Under urethane anesthesia (1.2g/kg subcutaneous injection), a PE50 catheter was placed into the bladder from the bladder dome for bladder filling and pressure recording. Intercontraction interval (ICI), bladder capacity (BC), pressure threshold (PT), maximum voiding pressure (MVP) and baseline pressure (BP) were measured during cystometry (continuous infusion of normal saline at rate of 0.04 ml/min).

Experimental Protocols: Atropine sulfate, tolterodine tartrate and propiverine hydrochloride were used as nonselective antimuscarinic receptor antagonists. Dimethindene maleate and methoctramine hemihydrate were used as M2-selective muscarinic antagonists. Darifenacin hydrobromide and 4-DAMP were used as M3-selective muscarinic antagonists. After baseline cystometry was recorded for 2 hr, vehicle (saline) or an antimuscarinic agent (30-60 μM for nonselective and M2-selective muscarinic antagonists or 150 μM for M3-selective muscarinic antagonists) was instilled intravesically for 30 min. Thereafter, 200 μM oxotremorine-M (Oxo-M; nonselective muscarinic agonist) was added to the infusate to examine whether intravesical application of Oxo-M with vehicle or an antimuscarinic agent induces bladder ovreactivity.

Statistical Analysis: An unpaired t test was used to compare cystometric parameters before and after intravesical application of Oxo-M with vehicle or an antimuscarinic agent. P<0.05 was considered significant.

Figure.1
Intercontraction interval



Results

a) When Oxo-M (200 μM) was instilled intravesically without antimuscarinic receptor antagonists, bladder ovreactivity was induced as evidenced by decreased ICI, BC and PT (788.6±74.1 to 469.0±43.0 sec, 0.56±0.04 to 0.41±0.04 ml and 6.93±0.40 to 5.75±0.35 cmH_2O, respectively). MVP or BP was not altered during Oxo-M instillation.

b) When instilled intravesically by itself, any of antimuscarinic agents (nonselective, M2-selective or M3-selective antagonists) did not change any cystometric parameters including MVP and BP.
c) When Oxo-M was instilled with nonselective or M2-selective antagonists, an Oxo-M-induced reduction in ICI, BC and PT was prevented. However, when Oxo-M was instilled with M3-selective antagonists, ICI and BC were significantly decreased in spite of the high dose application (150 μM) of M3-selective antagonists (darifenacin; 741.7±39.9 to 473.5.0±35.1 sec, 0.58±0.04 to 0.35±0.03ml; 4-DAMP; 674.6±72.8 to 413.1±41.0 sec, 0.57±0.04 to 0.37±0.03 ml, respectively). MVP or BP was not altered during instillation of Oxo-M with antimuscarinic agents.

Interpretation of results

a) Intravesical administration of Oxo-M activated local muscarinic receptors in the urothelium and/or afferent pathways to elicit bladder overactivity.
b) Intravesical application of antimuscarinic agents at a dose range of 30-150μM did not penetrate to the detrusor muscle layer to affect bladder contractility. In addition, because antimuscarinic drugs at these doses did not affect cystometric parameters by themselves, local muscarinic receptors in the urothelium and/or afferent pathways are not involved in normal micturition.
c) Bladder overactivity induced by intravesical administration of Oxo-M is mediated by the M2 muscarinic receptor subtype, but not the M3 muscarinic receptor subtype.

Concluding message

The M2 muscarinic receptor subtype plays an important role in the local cholinergic modulation of bladder afferent activity that contributes to bladder overactivity. Therefore, it is expected that antimuscarinic agents that have antagonistic activity against M2 receptors are more beneficial for the treatment of patients with overactive bladder.

References

1. Nat Clin Pract Urol (2007) 4; 46-54.
2. Auton Autacoid Pharmacol (2002) 3; 133-145.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Pittsburgh Institutional Animal Care and Use Committee**